Amendments to the Specification:

Please replace the title on page 1, line 5 with the following new title:

METHOD FOR TREATING IGE-MEDIATED DISORDERS

Please replace the paragraph on page 1, lines 9-11, with the following amended paragraph:

CROSS-REFERENCE TO RELATED APPLICATIONS

This is a continuation of U.S.S.N. 09/716,028, filed November 17, 2000, now allowed, which is a division of U.S.S.N. 09/109,207, filed June 30, 1998, now U.S.P. 6,172,213, which is a non-provisional application filed under 37 CFR 1.53(b), claiming priority under USC Section 119(e) to Provisional Application Serial No. 60/051,554 filed July 2, 1997, all of which are herein incorporated by reference.

Please replace the paragraph on page 3, lines 24-25 with the following amended paragraph:

In yet another embodiment, the present invention also relates to a method of treating and IgE-mediated disorder by the administration of the antibodies of the invention or functional fragments thereof.

Please replace the paragraph on page 3, lines 33-36 with the following amended paragraph:

Fig. 2 is a sequence comparison of the differences between the light chain and heavy chain CDR domains between rhuMabe25, e426, and sequences e26 and e27. The residue numbering here is consecutive, as opposed to that of Kabat *et al.* Also note that ~~the~~ these sequences are only fragments and not the actual full-length heavy and light chain residues.

Please replace the paragraph on page 4, lines 15-22 with the following amended paragraph:

Fig. 6 is a graphical representation of the affinity enrichment after various rounds of affinity selections described in part II of Example 4. The ratio of binding enrichment for each pool to that of the wild-type (Emut/Ewt) is displayed. The results indicate that the VL libraries (represented by "a" & "b") displayed successively improved relative enrichments, up to about 10-fold greater than wild-type after 5-6 rounds of enrichment. Moreover, the VH libraries "c" and "d"~~)~~ exhibited about a 3-fold improvement after around 3 rounds. Note that "a" corresponds to the Fab-phage library mutated at VL CDR-1 residues 27, 28, 30 and 31, while "b" corresponds to mutations at 30, 31, 32 & 34, while "c" and "d" are independent F(ab) libraries with mutations at residues 101, 102, 103, 105 & 107.

Please replace the paragraphs beginning on page 4, lines 29, and ending on page 5, line 4, with the following amended paragraphs:

Figs. 9A-C indicates the F(ab) apparent binding affinity of e25, e26 and e27, respectively, as measured by BIAcore TM-2000 surface plasmon resonance system. 1.5 serial dilutions of F(ab) antibody fragments were injected over the IgE chip in PBS/Tween buffer (0.05% Tween-20 in phosphate buffered saline) at 25°C using a flow rate of 20μl/min. The equilibrium dissociation constants (Kd) shown were calculated form the ratio of observed kon/koff for each Fab variant.

Figs. 10A-F ~~is~~are a sequence listing of the plasmid p426 which was used as the template for the construction of library-specific stop templates in Example 4.

~~Fig. 11. A. A~~Fig. 11A is a diagram of plasmid pDH188 insert containing the DNA encoding the light chain and heavy chain (variable and constant domain 1) of the Fab humanized antibody directed to the HER-2 receptor. VL and VH are the variable regions for the light and heavy chains, respectively. C_k is the constant region of the human kappa light chain. $CH1_{G1}$ is the first constant region of the human gamma 1 chain. Both coding regions start with the bacterial stII signal sequence.

~~B. A~~Fig. 11B is a schematic diagram of the entire plasmid pDH188 containing the insert described in 11A. After transformation of the plasmid into *E. coli* SR101 cells and the addition of helper phage, the plasmid is packaged into phage particles. Some of these particles display the Fab-p III fusion (where p III is the protein encoded by the M13 gene III DNA).

Please replace the paragraph at page 9, lines 24-39 with the following amended paragraph:

"Humanized" forms of non-human (*e.g.* murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', $F(ab')_2$ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibody may comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and optimize antibody performance. In general, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human

immunoglobulin and all or ~~subtantially~~ substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see: Jones *et al., Nature* 321, 522-525 (1986); Reichmann *et al., Nature* 332, 323-329 (1988) and Presta, *Curr. Op. Struct. Biol.* 2, 593-596 (1992).

Please replace the paragraph at page 10, lines 14-17, with the following amended paragraph:

The term "amino acid" and "amino acids" refer to all naturally ~~occuring~~ occurring L-α-amino acids. The amino acids are identified as hereinafter described under section A. Antibody Preparation: *(iv) Generation of mutant antibodies.* The term "amino acid variant" refers to molecules with some differences in their amino acid sequences as compared to a native amino acid sequence.

Please replace the paragraph at page 10, lines 27-30, with the following amended paragraph:

The term "cell", "cell line" and "cell culture" are used interchangeably, and all such designations include progeny. It is also understood that all progeny may not be precisely identical in DNA content, due to deliberate or inadvertent mutations. Mutant progeny that have the same function or biological property, as screened for in the ~~orginally~~originally transformed cell, are included.

Please replace the paragraph at page 10, lines 34-35, with the following amended paragraph:

"Transformation" means introducing DNA into an organism so that the DNA is replicable, either as an extrachromosomal element or by chromosomal ~~intergration~~.

Please replace the paragraph at page 11, lines 9-20, with the following amended paragraph:

The term "vector" means a DNA construct containing a DNA sequence which is operably linked to a suitable control sequence capable of effecting the expression of the DNA in a suitable host. Such control sequences include a promoter to effect transcription, an optional operator sequence to control such transcription, a sequence encoding suitable mRNA ribosome binding sites, and sequences which control the termination of transcription and translation. The vector may be a plasmid, a phage particle, or simply a potential genomic insert. Once transformed into a suitable host, the vector may replicate and function independently of the host genome, or may in some instances, integrate into the genome itself. In the present specification, "plasmid" and "vector" are sometimes used interchangeably, as the plasmid is the most commonly used form of vector at present. However, the ~~invneiton~~

invention is intended to include such other form of vectors which serve equivalent function as and which are, or become, known in the art. Typical expression vectors for mammalian cell culture expression, for example, are based on pRK5 (EP 307,247), pSV16B (WO 91/08291) and pVL1392 (Pharmingen).

Please replace the paragraph at page 13, lines 4-13 with the following amended paragraph:

The term "epitope tagged" when used herein refers to polypeptide fused to an "epitope tag." The epitope tag polypeptide has enough residues to provide an epitope against which an antibody thereagainst can be made, yet is short enough such that it does not interfere with activity of the polypeptide. The epitope tag preferably also is fairly unique so that the antibody thereagainst does not substantially cross-react with other epitopes. Suitable tag polypeptide generally have at least 6 amino acid residues and usually between about 8-50 amino acid residues (preferably between about 9-30 residues). Examples include the flu HA tag polypeptide and its antibody 12CA5 (Field *et al., Mol. Cell. Biol.* 8: 2159-2165 (1988)); the c-myc tag and the 8F9, 3C7, 6E10, G4, B7 and 9E10 antibodies thereagainst (Evan *et al., Mol. Cell. Biol.* 5(12): 3610-3616 (1985)); and the Herpes Simplex virus glycoprotein D (gD) tag and its antibody (Paborsky *et al., Protein Engineering* 3(6): 547-553 (1990)). In certain embodiments, the epitope ~~tage~~ tag is a "salvage receptor binding epitope."

Please replace the paragraph beginning at page 14, line 30 and ending at page 15, line 2 with the following amended paragraph:

The method provides a method for selecting novel binding polypeptides comprising: a) constructing a replicable expression vector comprising a first gene encoding a polypeptide, a second gene encoding at least a portion of a natural or wild-type phage coat protein wherein the first and second genes are heterologous, and a transcription regulatory element operably linked to the first and second genes, thereby forming a gene fusion encoding a fusion protein; b) mutating the vector at one or more selected positions within the first gene thereby forming a family of related plasmids; c) ~~tranforming~~ transforming suitable host cells with the plasmids; d) infecting the transformed host cells with a helper phage having a gene encoding the phage coat protein; e) culturing the transformed infected host cells under conditions suitable for forming recombinant ~~phagemide~~ phagemid particles containing at least a portion of the plasmid and capable of transforming the host, the conditions adjusted so that no more than a minor amount of phagemid particles display more than one copy of the fusion protein on the surface of the particle; f) contacting the phagemid particles with a target molecule so that at least a portion of the phagemid particles bind to the target molecule; and g) separating the phagemid particles that bind from those that do not. Preferably, the method further comprises transforming suitable ~~nost~~ host cells with recombinant phagemid particles that bind to the target molecule and repeating steps d) through g) one or more times.

Please replace the paragraphs beginning at page 15, line 12 and ending at page 15, line 15 with the following amended paragraph:

As used herein, "room" or "ambient ~~termperature~~temperature" shall be 23°C-25°C.

As used herein "binding polypeptide" means any polypeptide that binds with a selectable affinity to a target molecule. Preferably, the ~~poypeptide~~ polypeptide will be a protein that most preferably contains more than about 100 amino acid residues. Typically, the polypeptide will be a hormone or an antibody or a fragment thereof.

Please replace the paragraph beginning at page 16, line 30 and ending at page 17, line 6 with the following amended paragraph:

Also typically, the first gene will encode a mammalian protein, preferably, the protein will be an anti-IgE antibody. Additional antibodies are exemplified in section II.A. Antibody preparation, (vi) ~~*multipspecific*~~ *multispecific antibodies* (note however, that antibodies need not be multispecific). Additional polypeptides include human growth hormone (hGH), N-methionyl human growth hormone, bovine growth hormone, parathyroid hormone, thyroxine, insulin A-chain, insulin B-chain, proinsulin, relaxin A-chain, relaxin B-chain, prorelaxin, glycoprotein hormones such as follicle stimulating hormone (FSH), thyroid stimulating hormone (THS), and leutinizing hormone (LH), glycoprotein hormone receptors, calcitonin, glucagon, factor VIII, lung surfactant, urokinase, streptokinase, human tissue-type plasminogen activator (t-PA), bombesin, factor IX, thrombin, hemopoietic growth factor, tumor necrosis factor-alpha and -beta, enkephalinase, human serum albumin, mullerian-inhibiting substance, mouse gonadotropin-associated peptide, a microbial protein, such as betalactamase, tissue factor protein, inhibin, activin, vascular endothelial growth factor, receptors for hormones or growth factors, integrin, thrombopoietin, protein A or D, rheumatoid factors, nerve growth factors such as NGF-β, platelet-growth factor, transforming growth factors (TGF) such as TGF-alpha and TGF-beta, insulin-like growth factor-I and -II, insulin-like growth factor binding proteins, CD-4, DNase, latency associated peptide, erythropoietin, osteoinductive factors, interferons such as interferon-alpha, -beta and -gamma, colony stimulating factors (CSFs) such as M-CSF, GM-CSF and G-CSF, interleukins (Ils) such as IL-1, IL-2, IL-3, IL-4, superoxide dismutase, decay accelerating factor, viral antigen, HIV envelope proteins such as GP120, GP140, atrial natriuretic peptides A, B or C, immunoglobulins, and fragments of any of the above-listed proteins.

Please replace the paragraph at page 22, lines 17-26 with the following amended paragraph:

Between gene 1 and gene 2, DNA encoding a termination codon may be inserted, such termination codons are UAG (amber), UAA (ocher) and UGA (opel), *Microbiology*, Davis *et al.*, Harper & Row, New York, 1980, pp 237, 245-47 and 274). The termination codon expressed in a wild type host cell results in the synthesis of the gene 1

protein product without the gene 2 protein attached. However, growth in a suppressor host cell results in the synthesis of detectable quantities of fused protein. Such suppressor host cells contain a tRNA modified to inset an amino acid in the termination codon position of the mRNA thereby resulting in production of ~~detectible~~ detectable amounts of the fusion protein. Such suppressor host cells are well known and described, such as *E. coli* suppressor strain (Bullock *et al.*, *BioTechnologies* 5, 376-379 (1987)). Any acceptable method may be used to place such a termination codon into the mRNA encoding the fusion polypeptide.

Please replace the paragraph at page 26, lines 3-14 with the following amended paragraph:

Optionally the library of ~~phagmid~~ phagemid particles may be sequentially contacted with more than one immobilized target to improve selectivity for a particular target. For example, it is often the case that a ligand such as hGH has more than one natural receptor. In the case of hGH, both the growth hormone receptor and the prolactin receptor bind the hGH ligand. It may be desirable to improve the selectivity of hGH for the growth hormone receptor over the prolactin receptor. This can be achieved by first contacting the library of phagemid particles with immobilized prolactin receptor, eluting those with a low affinity (*i.e.* lower than wild type hGH) for the prolactin receptor and then contacting the low affinity prolactin "binders" or non-binders with the immobilized growth hormone receptor, and selecting for high affinity growth hormone receptor binders. In this case an hGH mutant having a lower affinity for the prolactin receptor would have therapeutic utility even if the affinity for the growth hormone receptor were somewhat lower than that of wild type hGH. This same strategy may be employed to improve selectivity of a particular hormone or protein for its primary function receptor over its clearance receptor.

Please replace the paragraph beginning at page 29, line 39 and ending at page 30, line 3 with the following amended paragraph:

The mammalian antibody selected will normally have a sufficiently strong binding affinity for the antigen. For example, the antibody may bind the human anti-IgE antigen with a binding affinity (Kd) value of no more than about 1×10^{-7} M, preferably no more than about 1×10^{-8} and most preferably no more than about 1×10^{-9} M. Antibody affinities may be determined by saturation binding; enzyme-linked ~~immunoabsorbant~~ immunosorbent assay (ELISA); and competition assays (*e.g.*, radioimmunoassays).

Please replace the paragraph beginning page 30, line 39 and ending at page 31, line 2 with the following amended paragraph:

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal antibodies directed against the antigen. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an *in vitro* binding assay, such as radioimmunoassay (RIA) or enzyme-linked ~~immunoabsorbent~~ immunosorbent assay (ELISA).

Please replace the paragraph at page 46, lines 19-23 with the following amended paragraph:

Examples of suitable ~~promotor~~ promoter sequences for use with yeast hosts include the promoters for 3-phosphoglycerate kinase or other glycolytic enzymes, such as enolase, glyceraldehyde-3-phosphate dehydrogenase, hexokinase, pyruvate decarboxylase, phosphofructokinase, glucose-6-phosphate isomerase, 3-phosphoglycerate mutase, pyruvate kinase, triosephosphate isomerase, phophoglucose isomerase, and glucokinase.

Please replace the paragraph at page 50, lines 23-35 with the following amended paragraph:

Stabilizers refer to a broad category of excipients which can range in function from a bulking agent to an additive which solubilizes the therapeutic agent or helps to prevent denaturation or adherence to the container wall. Typical stabilizers can be ~~polyhyric~~ polyhydric sugar alcohols (enumerated above); amino acids such as arginine, lysine, glycine, glutamine, asparagine, histidine, alanine, ornithine, L-leucine, 2-phenylalanine, glutamic acid, threonine, etc., organic sugars or sugar alcohols, such as lactose, trehalose, stachyose, mannitol, sorbitol, xylitol, ribitol, myoinisitol, galactitol, glycerol and the like, including cyclitols such as inositol; polyethylene glycol; amino acid polymers; sulfur containing reducing agents, such as urea, glutathione, thioctic acid, sodium thioglycolate, thioglycerol, α–monothioglycerol and sodium thio sulfate; low molecular weight polypeptides (*i.e.* < 10 residues); proteins such as human serum albumin, bovine serum albumin, gelatin or immunoglobulins; hydrophylic polymers, such as polyvinylpyrrolidone monosaccharides, such as xylose, mannose, fructose, glucose; disaccharides such as lactose, maltose, sucrose and ~~trisaccacharides~~ trisaccharides such as raffinose; polysaccharides such as dextran. Stabilizers are present in the range from 0.1 to 10,000 weights per part of weight active protein.

Please replace the paragraph at page 51, lines 5-8 with the following amended paragraph:

The formulation herein may also contain more than one active compound as necessary for the particular indication being treated, preferably those with complementary activities that do not adversely affect each other. For example, it may be ~~desireable~~ desirable to further provide an immunosuppressive agent. Such molecules are suitably present in combination in amounts that are effective for the purpose intended.

Please replace the paragraph at page 51, lines 31-40 with the following amended paragraph:

The amount of therapeutic polypeptide, antibody or fragment thereof which will be effective in the treatment of a particular disorder or condition will depend on the nature of the disorder or condition, and can be determined by standard clinical techniques. Where possible, it is ~~desireable~~ desirable to determine the dose-response curve and the pharmaceutical compositions of the invention first *in vitro*, and then in useful animal model systems prior to testing in humans. However, based on common knowledge of the art, a pharmaceutical composition effective in promoting the survival of sensory neurons may provide a local therapeutic agent concentration of between about 5 and 20 ng/ml, and, preferably, between about 10 and 20 ng/ml. In an additional specific embodiment of the invention, a pharmaceutical composition effective in promoting the growth and survival of retinal neurons may provide a local therapeutic agent concentration of between about 10 ng/ml and 100 ng/ml.

Please replace the paragraph at page 56, lines 1-17 with the following amended paragraph:

Fifteen days after the fusion, ~~supernatents~~ supernatants were tested for the presence of antibodies specific for human IgE using an enzyme-linked immunosorbent assay (ELISA). The ELISA was performed as follows, with all incubations done at room temperature. Test plates (Nunc Immunoplate) were coated for 2 hours with rat anti-mouse IgG (Boehringer Mannheim, #605-500) at 1 μg/ml in 50 mM sodium carbonate buffer, pH 9.6, then blocked with 0.5% bovine serum albumin in phosphate buffered saline (PBS) for 30 minutes, then washed four times with PBS containing 0.05% Tween 20 (PBST). Test supernatants were added and incubated two hours with shaking, then washed four times with PBST. Human IgE (purified from U266 cells as described above) was added at 0.5 μg/ml and incubated for one hour with shaking, then washed four times in PBST. Horseradish peroxidase conjugated goat anti-human IgE (Kirkegarrd & Perry Labs, #14-10-04, 0.5 mg/ml) was added at a 1:2500 dilution and incubated for one hour, then washed four times with PBST. The plates were developed by adding 100 μl/well of a solution containing 10 mg of *o*-phenylenediamine dihydrochloride (Sigma, #P8287) and 10 μl of a 30% hydrogen peroxide solution in 25 ml phosphate citrate buffer, pH 5.0, and incubating for 15 minutes. The reaction was stopped by adding 100 μl/well of 2.5 M sulfuric acid. Data was obtained by reading the plates in an automated ELISA plate reader at an absorbance of 490 nm. For MAE12, 365 supernatants were tested and 100 were specific for human IgE. Similar frequencies of IgE specificity were obtained when screening for the other antibodies. All of the monoclonal antibodies described herein were of the IgG1 isotype except for MAE17, which was IgG2b, and MAE14, which was IgG2a.

Please replace the paragraph at page 60, lines 21-29, with the following amended paragraph:

7. IgE *in vitro* Assay Protocol

a. Peripheral blood mononuclear cells were separated from normal donors.

b. Cells were washed extensively with PBS to remove as many platelets as possible.

c. Mononuclear cells were counted and resuspended in media at $1x10^6$ cells/ml. The media was a mixture of DMEM with penicillin and streptomycin, 15% horse serum, IL-2 (25 U/ml) and IL-4 (20 ng/ml).

d. Antibodies were added at appropriate concentrations on day 0, 5 and 8.

e. Cultures were incubated in 24 well Falcon tissue culture plates for 14 days.

f. On day 14, supernatants were removed and assayed for IgE concentrations by an IgE specific ELISA ~~potocol~~protocol.

Please replace the paragraph at page 61, lines 22-37, with the following amended paragraph:

The murine anti-human IgE mAb MaE11, shown in Figure 1, was modified by site-directed mutagenesis (Kunkel, T.A. (1985), *Proc. Natl. Acad. Sci. USA* 82: 488) from a deoxyuridine-containing template containing a human *k*-subgroup I light chain and human subgroup III heavy chain (VH-CH1) in a pUC119-based plasmid, pAK2 (Carter *et al.* (1992), *Proc. Natl. Aca. Sci. USA* 89: 4285) to obtain the variant F(ab)-1. F(ab)-2 was constructed from the F(ab)-1 template and all other humanized F(ab) variants were constructed from a template of F(ab)-2. The plasmids were transformed into *E. coli* strain JM101 for the preparation of double- and single stranded DNA (Messing, J. (1979), *Recomb. DNA Tech. Bull.* 2: 43; ~~Ausuble~~ Ausubel *et al.*, *Current Protocols in Molecular Biology*, Unit 1 (1997)). Both the light and heavy chain residues were completely sequenced using the dideoxynucleotide method. The DNA encoding light and heavy chains was then subcloned into a derivative of the *E. coli* F(ab) expression plasmid, pAK19 (Carter *et al.* (1992), *Biotechnology* 10: 163). The derivative lacked the hinge ~~cyteines~~cysteines that form the inter~~heavy~~-chain disulfides in $F(ab')_2$ fragments. The F(ab) fragments, as opposed to full-length IgG antibodies, facilitated the analysis of a moderately large number of variants because *E. coli* expression could be used rather than mammalian cell culture techniques. These individual variants are described in application WO 93/04173 published 4 March 1993. Once the best variant was determined, it was subsequently subcloned into a plasmid encoding a full-length human IgG1 (see below).

Please replace the paragraph beginning on page 61, line 38 and ending on page 62, line 7 with the following amended paragraph:

The expression plasmids were transformed into *E. coli* strain MM294 (Meselon, M and R. Yuan (1968), *Nature* 217: 1110), and a single colony was grown in 5 ml of 2YT media-carbenicillin (100 µg/ml) for 5-8 hours at 37°C. The culture (5 ml) was then added to 100 ml of AP5 media-carbenicillin (100 µg/ml) and allowed to grow for 16 hours in a 500 ml shaker flask at 37°C. The culture was centrifuged at 4,000 x g and the ~~supernatent~~ supernatant removed. After freezing for 1 hour, the pellet was resuspended in 5 ml cold 10 mM Tris, 1mM EDTA and 50 µl 0.1 M benzamidine (Sigma, St. Louis), the latter of which was added to inhibit proteolysis. After gentle shaking on ice for 1 hour, the sample was centrifuged at 10,000 x g for 15 minutes. The supernatant was applied to a protein A-Sepharose CL-4B (Pharmacia) column (0.5 ml bed volume) then washed with a 10 ml solution of 3 M potassium chloride/100 mL Tris, pH 8.0, followed by elution with 100 mM acetic acid (2.5 ml), pH 2.8 into 1 M Tris, pH 8.0 (0.5 ml).

Please replace the paragraph at page 63, lines 6-15 with the following amended paragraph:

FACS-based binding assays:

The ability of the antibody to inhibit FITC-conjugated (Goding, J.W. (1976), *J. Immunol. Methods* 13: 215) IgE binding to the α-chain of the high-affinity FcεRI receptor expressed on CHO 3D10 cells (Hakimi, J. *et al.* (1990), *J. Biol Chem.* 265: 22079) was determined by flow cytometry. FITC-conjugated IgE (40 nM) was preincubated with the antibody (0.3-1.0 x 10^{-6} M) at 37°C for 30 minutes in FACS buffer (PBS, 0.1% BSA, and 10 mM sodium azide, pH 7.4). The complex was then incubated ~~wth~~ with 5 x 10^5 CHO CD10 cells at 4°C for 30 minutes. The cells were washed three times with FACS buffer and mean channel fluorescence at 475 nm measured on ~~an~~ a FACScan flow cytometer (Becton Dickinson). MaE1, a murine anti-human IgE mAb that does not block IgE binding to the FcεRI α-chain, was used as a positive control and MOPC21 (Cappel), a murine monoclonal that does not recognize IgE, was used as a negative control. The results are described in Figure 3.

Please replace the paragraph at page 64, lines 31-34 with the following amended paragraph:

To test the effects of buried residues on CDR conformation, F(ab)-3 to F(ab)-7 were constructed in which murine residues were changed back to human ones. As is indicated in Table 4 (by F(ab)-3 & F(ab)-4), the side chains at VL4 and VL33 have minimal effect on binding and ~~presumeably~~ presumedly the conformation of CDR-L1 in the humanized antibody.

Please replace the paragraph at page 65, lines 5-8 with the following amended paragraph:

F(ab)-10, which displayed substantially improved binding relative to F(ab)-2, was a variant in which all buried residues (defined as residues with ~~accesible~~ accessible surface area being less than 5% of that of the free amino acid) in both the VL and VH domains were those of murine MaE11. In essence, F(ab)-10 can be thought of as murine MaE11 in which only exposed, non-CDR residues in VL and VH were changed to human residues.

Please replace the paragraphs beginning at page 67, lines 9 and ending page 68, line 3 with the following amended paragraph:

Binding of ~~MeE11~~MaE11 to ~~to~~ IgE-loaded FcεRI:

Murine MaE11 prevents binding of free IgE to FcεRI on mast cells but does not trigger histamine release by binding to IgE-loaded FcεRI. As shown in Fig. 4, both murine MaE11 and humanized variant 12 (IgG1-12) as well as the negative isotype control antibody MOPC21 and the negative isotype control humanized 4D5 (Carter *et al.*, *supra*) did not bind IgE-loaded FcεRI on CHO 3D10 cells. In contrast, the murine MaE1 antibody, which binds to IgE but does not prevent IgE binding to FcεRI, bound to the IgE-loaded FcεRI. Unlike the human IgG1 control (humanized 4D5), the murine IgG1 isotype (as represented by MOPC21) exhibits a nonspecific background binding of approximately 10% on these cells. MaE11 did not give staining above the MOPC21 control and humanized variant 12 did not give staining above the humanized 4D5 control (Fig. 4).

Partial alanine scanning of CDR residues important in IgE binding:

The sequences of the MaE11 CDR's indicate a preponderance of charged residues (Fig. 1). CDR-L1 contains three Asp residues, whereas CDR-L3 possesses His, Glu and Asp. CDR-H3 has three His residues. The models of murine and humanized MaE11 illustrated the spatial proximity of the all of these charged residues (not shown). In contrast, the lone Asp 54 in CDR-H2 is spatially ~~sparated~~ separated from the other charged residues. Alanine was substituted, by site-directed mutagenesis (Kunkel, T.A. (1985), *Proc. Natl. Acad. Sci. USA* 82: 488), for each of these charged residues to generate variants. In CDR-L1, alteration of one of the three Asp residues, Asp VL32b, effectively abolished IgE binding [F(ab)-16; Table 5], whereas substitution of the other Asp residues had minimal effect [F(ab)-14; F(ab)-15]. Simultaneous alteration of Glu VL93 and Asp VL94 to alanine in CDR-L3 [Fa(ab)-17; Table 5], also reduced binding, although not to the same extent as did replacement at VL32b. Individual substitution of the three His residues in CDR-H3 with Ala resulted in either slightly improved binding [F(ab)-21] or a three-fold reduction in binding [F(ab)-20 & F(ab)-22]. However, simultaneous alteration of all three His residues abolished binding [F(ab)-19]. Although it is not readily determinable whether the charged residues are involved in direct binding to IgE or to provide some conformational stability to their respective CDR's, variants F(ab)-13 to F(ab)-22 show that CDR-L1 and CDR-H3 are important determinants in IgE binding.

Please replace the paragraphs beginning on page 71, line 18 and ending on page 71, line 39 with the following amended paragraph:

I. Construction of monovalent F(ab)-phage libraries

Several F(ab) libraries were constructed. As a starting vector, an e25 variant containing the VL substitution D32E (to eliminate IsoAsp isomerization) was fused to the C-terminal domain of bacteriophage M13g3p by known techniques, see for example Bass *et al.*, *Proteins* 8: 309 (1990). This plasmid, which was known as p426 appears in Fig. 10. First, the "wild-type" F(ab)-phage, p426 was used as the template for construction of library-specific "stop" templates. By introducing stop codons (TAA or TGA), the original molecule is rendered inactive, ~~therey~~ thereby reducing background effects and template-specific (hybridization) bias in the mutagenesis steps for constructing the library (Lowman & Wells, *Methods: Comp. Methods Enzymol.* 3: 205 (1991)). These templates were constructed using single-stranded template-directed mutagenesis (Kunkel *et al.*, *Methods Enzymol.* 204: 125 (1991)), with the oligonucleotides listed in Table 10.

Subsequently, these stop-templates were used in a second round of mutagenesis, using the oligos listed in Table 11, to generate libraries in each of the indicated CDR regions. NNS degenerate codons were used to yield all twenty amino acids in each of the indicated CDR regions. (Nucleotide bases are indicated in single-letter IUPAC nomenclature; N = A, G, C or T; S = G or C). NNS degenerate codons were used to yield all twenty amino acids at

each randomized positions, using 32 different possible codons. An amber stop codon (TAG) encodes Gln in the suppressor system used here; *i.e.*, the *sup*E ~~supressor~~ suppressor strain XL-1 Blue; Bullock *et al. Biotechniques* 5, 376 (1987). The presence of an amber codon between the heavy-chain antibody domain and the g3p domain on phage permits the expression of the phage-displayed fusion protein only in amber suppressor strains of *E. coli*, while soluble F(ab) protein can be obtained with this same construct in non-~~supressor~~ suppressor strains of *E. coli*. (Lowman *et al. Biochemistry* 30: 10832 (1991); Lowman and Wells, *Methods Comp. Methods. Enzymol.* 3: 205 (1991); Hoogenboom *et al., Nucl. Acids Res.* 19: 4133 (1991). However, other stop codons for use in other *E. coli* phage expression systems are apparent to those of ordinary skill in the art.

Please replace Tables 10 and 11 appearing at page 72 with the following amended Tables:

Table 10

Stop-Template Oligos for First-Round Mutagenesis

Oligo sequence no.	Region	Sequence
HL-208	VL1	ACC TGC CGT GCC AGT TAA TAA GTC TAA TAA GAA GGT GAT AGC TAC (SEQ ID NO:27)
HL-209	VH3	GCC AGT CAG AGC GTC TAA TAA TAA GGT TGA AGC TAC CTG AAC TGG T (SEQ ID NO:28)
HL-210	VH3	TGT GCT CGA GGC AGC TAA TAA TAA GGT TAA TGG TAA TTC GCC GTG TGG GG (SEQ ID NO:29)
HL-220	VL2	G AAA CTA CTG ATT TAC TAA TAA TAA TAA CTG GAG TCT GGA GTC (SEQ ID NO:30)
HL-221	VL3	CT TAT TAC TGT CAG CAA AGT TAA TAA TAA CCG TAA ACA TTT GGA CAG GGT ACC (SEQ ID NO:31)
HL-222	VH1	G TCC TGT GCA GTT TCT TAA TAA TAA TAA TAA TCC GGA TAC AGC TGG (SEQ ID NO:32)
HL-223	VH1	GCC TAC TCC ATC ACC TAA TAA TAA AGC TGA AAC TGG ATC CGT CAG (SEQ ID NO:33)
HL-224	VH2	GG GTT GCA TCG ATT TAA TAA TAA GGA TAA ACT TAA TAT AAC CCT AGC CTC AAG (SEQ ID NO:34)
HL-225	VL1	AAG CCG GTC GAC AGG TAA TAA GAT TAA TAC TAA AAC TGG TAT CAA CAG (SEQ ID NO:35)

Table 11

Library-Specific, Degenerate Oligos for Second Round Mutagenesis

HL-212	VL1	ACC TGC CGT GCC AGT NNS NNS GTC NNS NNS GAA GGT GAT AGC TAC (SEQ ID NO:36)
HL-213	VH3	GCC AGT CAG AGC GTC NNS NNS NSS GGT NNS AGC TAC CTG AAC TGG (SEQ ID NO:37)
HL-214	VH3	TGT GCT CGA GGC AGC NNS NNS NNS GGT NNS TGG NNS TTC GCC GTG TGG GG (SEQ ID NO:38)
HL-231	VL2	G AAA CTA CTG ATT TAC NNS NNS NNS NNS CTG GAG TCT GGA GTC (SEQ ID NO:39)
HL-232	VL3	CT TAT TAC TGT CAG CAA AGT NNS NNS NNS CCG NNS ACA TTT GGA CAG GGT ACC (SEQ ID NO:40)
HL-233	VH1	G TCC TGT GCA GTT TCT NNS NNS NNS NNS NNS TCC GGA TAC AGC TGG (SEQ ID NO:41)
HL-234	VH1	GTT TCT GGC TAC TCC ATC ACC NNS NNS NNS AGC NNS AAC TGG ATC CGT CAG (SEQ ID NO:42)
HL-235	VH1	GG GTT GCA TCG ATT NNS NNS NNS GGA NNS ACT NNS TAT AAC CCT AGC GTC AAG (SEQ ID NO:43)
HL-236	VL1	AAG CCG GTC GAC AGG NNS NNS GAT NNS TAC NNS AAC TGG TAT CAA CAG (SEQ ID NO:44)

Please replace the paragraph at page 73, lines 10-13 with the following amended paragraph:

Maxisorp 96-well ~~platic~~ plastic plates (Nunc) were coated with 2 μg/ml IgE (human IgE; Genentech lot #9957-36) in 50 mM sodium carbonate buffer, pH 9.6, overnight at 4°C. The IgE solution was then removed, and the plates were incubated with a blocking solution of horse serum (without Tween™-20), for 2 hours at ambient temperature.

Please replace the paragraph at page 79, lines 5-6 with the following amended paragraph:

Conclusion: The plots in Fig. 8 indicate that both E26 and E27 have greater ~~affintity~~ affinity than E25 for the high affinity receptor and that E27 showed the greatest affinity.

Please replace the paragraph at page 80, lines 1-15 with the following amended paragraph:

VIII. F(ab) Expression and Purification:

Anti-IgE F(ab) E-25 (Presta *et al.* , *J. Immunol.* 151: 2623-2632 (1993)) and variants in phagemids derived from p426 (Fig. 10) were expressed in *E. coli* strain 34B8. Toothpick cultures (10 ml) in 2YT media with 50 μg/ml carbenicillin were incubated 8 hours at 37°C and then transferred to 1 liter of modified AP-5 containing 50 μg/ml carbenicillin and incubated for 24 hours at 37°C. Cultures were centrifuged in 500 ml bottles at 7,000 rpm for 15 minutes at 4°C. The pellet was frozen for at least 3 hours at -20°C. Each 500 ml pellet was suspended in 12.5 ml cold 25% sucrose in 50 mM Tris pH 8.0 containing 1 mM benzamidine (Sigma) at 4°C. Suspension was solubilized by stirring at 4°C for 3 hours. Suspension was centrifuged at 18,000 rpm for 15 minutes at 4°C and the F(ab)s expressed in the supernatant were purified by protein G (Pharmacia) affinity chromotography. The column was washed with a solution of 10 mM Tris (pH 7.6) and 1 mM EDTA (pH 8.0) and the F(ab)s were eluted with 2.5x column volumes of 100 mM acetic acid (pH 3.0) and immediately returned to neutral pH with 0.5 volumes of 1M Tris pH 8.0. Eluates were concentrated and buffer exchanged against PBS with centricon 30 microcentrators (Amicon). Protein ~~concenraion~~ concentration was determined by absorbance at 280 nM with a spectrophotometer (Beckman DU 64) and sample purity was evaluated using 4-20% SDS PAGE gels (Novex) under reducing conditions with 5% β-mercaptoethanol.

Please replace the original sequence listing, appearing at pages 81-106 of the original parent specification, U.S.S.N. 09/109,207, and replace it with the sequence listing filed in the immediate parent application, U.S.S.N. 09/716,028 (copy enclosed).